Total Phenolic, Flavonoids, Tannin Content and Antioxidant Power of Some Iranian Pomegranate Flower Cultivars (*Punica granatum* L.)*

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Received July 10th, 2013; revised August 10th, 2013; accepted August 25th, 2013

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ABSTRACT

Recently, pomegranate (*Punica granatum* L.) was demonstrated to be high in antioxidant activity and strong in phenolic, flavonoid and tannin contents in its fruit, flower and also aerial part. In this paper six cultivars of Iranian pomegranate flower including Ghojagh, Rabbab, Malas, Shishegap, Danesiah and Golnar have been investigated. The maximum amount of total phenolic was detected in Ghojagh (25.94 mg·GAEg⁻¹) and flavonoid showed the highest content in Danesiah (23.06 mg·CEg⁻¹). The lowest content of these two groups was observed in Golnar (15.19 mg·GAEg⁻¹ and 11.46 mg·CEg⁻¹). Measurement of tannin compounds showed that Rabbab by 2.03% and Golnar by 1.06% have the highest and lowest amount respectively. According to the FRAP method, Ghojagh and Golnar have the highest and lowest antioxidant values respectively (452.53 mmol·g⁻¹ and 123.39 mmol·g⁻¹). As a result of HPLC-DPPH method, Malas and Danesiah have the highest and lowest antioxidant value (116.38 and 97.64 mgVEEg⁻¹).

Keywords: Pomegranate; Flower; Antioxidant; Phenolic; HPLC

1. Introduction

Pomegranate (*Punica granatum* L.) which is widely cultivated in Iran has been popular worldwide over the years originated from Middle East and Iran [1,2]. The pomegranate fruit has been commercialized and can be found as juice, jellies, wine. The wide adoption of the pomegranate is due to the recent studies that mentioned it that contains a high amount of antioxidants which are beneficial to our health in many ways [3-6]. Its great flavor and health benefits have made it a great candidate for those who search natural healthy foods [7]. Pomegranate is an important source of bioactive compounds and different parts of it have been used in medicine for many centuries [3,8] and the edible parts used pharmaceutically worldwide. In tradition medicine, the pricarp was used by Chinese for the treatment of diarrhea, metrorrhagia, metrorstaxis and bellyache. The flower was used as a flower supplement to treat diabetes mellitus in Unani medicine and the diarrhea was treatment by pomegranate fruit in South Africa [9]. Pomegranate juice has been demonstrated to be high in antioxidant activity and is effective in the prevention of atherosclerosis, coronary heart disease and cancer [4,10]. There are some reports about the presence of tannins, alkaloids, glycosides, flavonoids and phenolic compounds as antioxidant factors in juice, peel, pulp, and seed fractions of pomegranate [11-13]. In the case of flower, the pomegranate flowers had a medicinal use in Traditional Iranian Medicine and also in the current studies [14,15]. In folk medicine the decoction of flowers is used to stop bleeding and purging [16,17].  

*The authors declare that they have no conflict of interests.

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enhanced acute glucose-stimulated insulin secretion at basal and stimulatory glucose concentrations in pancreatic b-cell. Such effects may contribute to the antidiabetic properties [21]. The bright colour of pomegranate flowers and arils is due to anthocyanins [22]; however, only one anthocyanin compound (i.e. pelargonidin-3,5-diglucoside) has yet been identified in pomegranate flowers using HPLC [23], whereas in pomegranate juice, princepally cyanidin-3-O-glucoside, cyanidin-3,5-di-O-glucoside, delphinidin-3-O-glucoside, delphinidin-3,5-di-O-glucoside, pelargonidin-3-O-glucoside, and pelargonidin-3,5-di-O-glucoside, have been reported [24, 25]. Oleanolic acid, ursolic acid and gallic acid, active components contained in pomegranate flower [26], have long been recognized to have antihyperlipidemic properties [27,28]. It is known that the amount of organic acids, phenolic compounds, sugars, water-soluble vitamins, and minerals of all parts of pomegranates are different in various researches which may be attributed to their cultivar origins [29,30].

Therefore, in this study, the contents of total phenolic, flavonoids, and tannins of the some Iranian pomegranate flower cultivars and their antioxidant activity were investigated through the FRAP and HPLC-DPPH methods.

2. Materials and Methods

2.1. Sample Preparation

Six cultivars of pomegranate’s flower were obtained from Agricultural Research Center, Yazd, Iran. The flowers varieties (Malas, Shishegap, Danesiah, Rabbab, Ghojagh and Golnar) were harvested during May 2012 from different mature trees which randomly selected. Flowers were desiccated in shade and room temperature. Then, different flower cultivars were grounded separately by different mature trees which randomly selected. Flowers were desiccated in shade and room temperature. Then, different flower cultivars were grounded separately by mortar. 0.5 g of each cultivar was shaken with methanol 80% for 2 hours and centrifuged in 10,000 rpm [Hereus-Germany] then the extracts were separated and stored in 4°C [9].

According to the tannin determination, 3 g of each dried flower powder was extracted with deionized distilled water in 250 mL volumetric flask during 4 hours at room temperature and then the sample was filtered [31].

2.2. Total Phenolic Content

Total phenolics contents were determined according to the Folin-Ciocalteu method with slight modifications [32]. The extract (200 μL) was mixed with 1.5 mL of Folin-Ciocalteu reagent [previously diluted 10 times with double distilled water] and allowed to stand at room temperature for 5 min. 1.5 mL sodium bicarbonate solution [60 g L⁻¹] was added to the mixture and after incubation for 90 min at room temperature, the absorbance level was measured at 725 nm using a UV-Visible spectrophotometer (GBC, Cintra 40). Total phenolic were quantified by calibration curve obtained from measuring the absorbance of the known concentrations of gallic acid standard solutions [10 - 150 μg·mL⁻¹ in 80% methanol]. The results were calculated as gallic acid equivalent (GAE) per one gram dry powder and reported as mean value ± standard deviation (SD).

2.3. Total Flavonoid Content

Total flavonoid content was measured by the aluminum chloride colorimetric method [33]. An aliquot (1 mL) of each extract was added to 10 mL volumetric flask containing 4 mL of double distilled water. Then 0.3 mL NaNO₂ 5% was added to the flask and after 5 min, 0.3 mL AlCl₃ [10%] was also added. At 6th min, 2 mL NaOH (1 M) was added and the total volume was made up to 10 mL with double distilled water. The solution was mixed completely and the absorbance level was measured versus prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg catechin equivalents (CE) per one gram dry powder. The total flavonoid assay was measured three times for each pomegranate extract. 1 mL of standard solution (catechin: 5 - 100 mg/L) was used to construct calibration curve.

2.4. Antioxidant Assay (FRAP Method)

The FRAP (Ferric reducing antioxidant power) assay was described initially by Benzie and Strain [34]. The principle of this method is based on the reduction of the ferric-tripryridyl triazine complex to its ferrous colored form in the presence of antioxidants. Briefly, the FRAP reagent contained 5 mL TPTZ (2,4,6-tripyridyl-S-triazine, 10 mmol·L⁻¹) solution in 40 mmol·L⁻¹ HCl plus 5 mL FeCl₃ [20 mol·L⁻¹] and 50 mL of Acetate buffer (0.3 mol·L⁻¹). It was prepared freshly and set at 37°C. Aliquots of 50 μL sample supernatant were mixed with 1.5 mL FRAP reagent and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37°C for 10 min. To construct the calibration curve five concentrations of FeSO₄·7H₂O (100 - 1000 mmol·L⁻¹) were used and the absorbencies were measured as sample solution. The values were expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol·L⁻¹ FeSO₄. All the measurements were taken in triplicate and expressed as mean value ± RSD.

2.5. Antioxidant Assay [HPLC-DPPH Method]

The chromatographic analysis was carried out by a Knaus HPLC system [Berlin, Germany] equipped with an auto-sampler, pump and a UV–Vis detector. 20 μL of each samples (1 mL extract was volumed to 5 mL with methanol 80% in volumetric flask) was added to 2 mL
1,1-diphenyl-2-picrylhydrazyl [DPPH] solution at a concentration of 0.1 mmol·L⁻¹ and mixed with 20 mL deionized distilled water. Trolox (1 mg·mL⁻¹) and deionized distilled water were used as the standard of vitamin E and blank respectively and were prepared by adding 20 µL of each one to 2 mL DPPH solution. The mixture was shaken 20 seconds and then kept in the darkness 40 min at room temperature. 20 µL of prepared samples which were filtered through 0.2 µm membrane filter [Control Biogen-Spain] was injected to the HPLC. The radical scavenging activity of DPPH was measured at 517 nm. All samples were analyzed in triplicate (mean ± RSD) [35].

2.6. Tannin Assay

The analyses of tannin content in flowers were performed according to the International Pharmacopoeia and AOAC methods [36] with some modifications. 3 g of flower powder was infused with 250 mL of deionized double distilled water and then it was filtered through 0.45 µm (Control Biogen-Spain) sample filter. 25 mL of the infusion was added into 1 L conical flask and then 25 mL of indigo solution [0.6%] and 750 mL deionized distilled water was added. The solution has been titrated with 0.1 N aqueous solution of KMNO₄ until the blue colored solution changed to golden yellow one. Standard solution of indigo carmine was prepared as following: 6 g indigo carmine was dissolved in 500 mL of deionized distilled water by heating and after cooling 50 mL of 98% H₂SO₄ was added. The solution was diluted to 1 L with deionized distilled water and then it was filtered through 0.2 µm membrane filter. The blank test was carried out by titration of the mixture of 25 mL indigo carmine and 775 mL double distilled water. All samples were analyzed in duplicates. The tannin percent [%] in the samples were calculated as follows:

\[ T(\%) = \frac{[V - V₀]}{V₀} \times 100 \times \frac{g}{25} \times \frac{100}{g} \times \frac{1}{25} \]

where V is the volume of 0.1 N aqueous solution of KMNO₄ used in the titration of the sample and V₀ is the volume of 0.1 N aqueous solution of KMNO₄ used in the titration of the blank sample as mL; 0.004157 is the tannins equivalent in 1 mL of 0.1 N aqueous solution of KMNO₄; g is the mass of the sample taken for the analysis as gram and 250 is the volume of the volumetric flask.

2.7. Statistical Analysis

Three replicates of each sample were used for statistical analysis and the values were reported as mean ± RSD. Pearson’s correlation was carried out using SPSS statistical program to study the relationship between antioxidant activity and total phenolic and flavonoid content. Data were also subjected to the analysis of variance and mean values were compared by Tukey post-hoc multiple comparison test. Differences at p-value <0.05 were considered to be significant.

3. Results and Discussion

Pomegranate flower has been used in traditional Iranian medicine according to its medicinal effects [14]. The total phenolic content of pomegranate flower extracts is expressed in term of gallic acid equivalent (the standard curve equation: \( Y = 0.005X - 0.0234, r² = 0.9975 \)). It was ranged from 25.94% to 15.19% mg gallic acid equivalents per gram of dry powder in Ghojagh and Golnar respectively (Table 1). The total flavonoid content of flower extracts is also expressed in terms of catechin equivalent (the standard curve equation: \( y = 0.005x + 0.1478, r² = 0.9919 \)), ranged from 23.06% to 11.46% mg catechin equivalents per gram of dry flower powder in Danesiah and Golnar respectively (Table 1).

Phenolic and flavonoid contents are important in antioxidant power of herbs and the analysis of their amount in different pomegranate flower cultivars via ANOVA shows that Ghojagh has the most amount of total phenol and Golnar has the least ones (p-value < 0.05). In the case of total flavonoid, Danesiah cultivar has the most content (23.06 mgCE·g⁻¹) and Golnar cultivar has the

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Total phenol [mg GAE/g dry powder ± RSD]</th>
<th>Total flavonoid [mg CE/g dry powder ± RSD]</th>
<th>Total tannin [% ± RSD]</th>
<th>Flavonoid/Phenolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghojagh</td>
<td>25.94a ± 7.00</td>
<td>19.17b ± 4.31</td>
<td>1.33b ± 0.22</td>
<td>0.74c</td>
</tr>
<tr>
<td>Rabbab</td>
<td>24.57a ± 5.04</td>
<td>16.76c ± 2.17</td>
<td>2.03 ± 0.15</td>
<td>0.68b</td>
</tr>
<tr>
<td>Shishegap</td>
<td>20.60a ± 6.21</td>
<td>18.30a ± 2.32</td>
<td>1.29a ± 0.43</td>
<td>0.89b</td>
</tr>
<tr>
<td>Danesiah</td>
<td>23.48b ± 4.46</td>
<td>23.06c ± 3.46</td>
<td>1.98b ± 0.67</td>
<td>0.98b</td>
</tr>
<tr>
<td>Malas</td>
<td>18.68b ± 3.01</td>
<td>18.21b ± 4.49</td>
<td>1.47c ± 0.44</td>
<td>0.97c</td>
</tr>
<tr>
<td>Golnar</td>
<td>15.19b ± 2.02</td>
<td>11.46c ± 2.17</td>
<td>1.06c ± 0.11</td>
<td>0.75c</td>
</tr>
</tbody>
</table>

Values in the same column bearing different superscripts are significantly (p ≤ 0.05) different.

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Table 2. Antioxidant power of six pomegranate flower cultivar according to the FRAP and HPLC-DPPH method.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>mmol Fe$^{2+}$ equivalent/g dry powder ± RSD</th>
<th>Mg vitamin E equivalent/g of dry powder ± RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghojagh</td>
<td>452.53 ± 25.08</td>
<td>109.93 ± 20.60</td>
</tr>
<tr>
<td>Rabbab</td>
<td>219.77 ± 19.87</td>
<td>112.13 ± 14.40</td>
</tr>
<tr>
<td>Shishegap</td>
<td>219.05 ± 13.18</td>
<td>109.68 ± 16.48</td>
</tr>
<tr>
<td>Danesiah</td>
<td>200.33 ± 21.44</td>
<td>97.64 ± 16.69</td>
</tr>
<tr>
<td>Malas</td>
<td>337.04 ± 15.45</td>
<td>116.38 ± 20.81</td>
</tr>
<tr>
<td>Golnar</td>
<td>123.39 ± 17.38</td>
<td>107.63 ± 21.02</td>
</tr>
</tbody>
</table>

Values in the same column bearing different superscripts are significantly ($p \leq 0.05$) different.

least content (11.46 mgCE·g$^{-1}$) significantly. Analysis of antioxidant power according to the FRAP method show Ghojagh (452.53 mmol Fe$^{2+}$·g$^{-1}$) has the most antioxidant power while Golnar (123.39 mmol Fe$^{2+}$·g$^{-1}$) has the least ones. By the HPLC-DPPH method Malas (116.38 mgVitE·g$^{-1}$) have the most antioxidant effect and Danesiah (97.64 mgVitE·g$^{-1}$) has the least antioxidant power (Table 2). The Pearson’s correlation showed no significant correlation between total phenolic and flavonoid content and antioxidant power via both FRAP and HPLC-DPPH methods. The total tannin content in different cultivars were also compared according to the statistical calculation and the results showed Rabbab and Danesiah cultivars have the highest amount of tannin and Golnar has the least content. The flavonoid-phenolic ratio in Table 1 is mentioned to show the importance of flavonoids in total phenolic content and its antioxidant activity. The range of this ratio is between 0.98 in Danesiah and 0.68 in Rabbab.

In a prosperous in vitro and also in vivo study by Kaur et al. [15] the pomegranate flower extract indicated a significant antioxidant activity and it was found to exhibit a potent protective role in acute oxidative tissue injury animal in vivo model. Also the ethanolic extract of pomegranate flower showed 81.6% antioxidant activity in DPPH model system.

Comparison of the pomegranate flower results with its pulp and peel [37] showed flowers have higher amount of the total phenol and flavonoids content, but it has less antioxidant activity according to the FRAP method. It can be suggested that water soluble antioxidant such as organic acid can leads to antioxidant activity of pulp and water insoluble component to the flower. In another study the antioxidant activity and total phenolic content of pomegranate flower and juice were compared and in spite of higher amounts of phenolic compounds in flower extracts, antioxidant activity of juices were more than flowers indicating results as the same as this study [38]. Total phenolic and flavonoid content of peel and its antioxidant activity in another research is remarkable more than the flower and suggest the peel as a better source of antioxidant components [37]. In Orak et al. study [39], the DPPH scavenging activity of antioxidant in juice, peel, and seed parts of pomegranate were investigated. The results showed that the EC$_{50}$ value of DPPH scavenging activities in peel extracts was 23.4-fold higher than the juice extracts, and the seed extracts had 2.3-fold higher than juice. Also the reducing power in peel extracts was found to be 4.7-fold higher than seed extracts and 10.5-fold higher than the juice. The data expressed that, in peel and pulp except that, the total polyphenol and tannin contents, flavonoid and anthocyanin play an important role in antioxidant activity respectively.

4. Conclusion

The antioxidants assessment suggests that the studied pomegranate flower and its associated bioactive compounds such as phenolic, flavonoids and tannins compounds may possess a strong potential as a chemo preventive and possibly as new tools for preventing various human diseases. However, further studies should focus on developing the novel pomegranate derived products such as ready-to-eat pomegranate flower, single-strength juices, flower extract concentrates, flower in syrup, and the frozen flower, to benefit from these constituents throughout a healthy life cycle.

5. Acknowledgements

This work was student thesis and supported by the grant from the research council of Tehran University of Medical Sciences, Tehran, Iran.

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